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Mechanisms behind the inhibition of autotrophic nitrification following rice-straw incorporation in a subtropical acid soil

Yushu Zhang[a,b,c], Xiangzhou Zheng[a], Baoling Guo[a], Juhua Yu[a], Alison Carswell[c], Tom Misselbrook[c], Jinbo Zhang[d], Christoph Müller[e,f], Deli Chen[b], Hong Ding[a,*]

[a] Institute of Soil and Fertilizer, Fujian Academy of Agricultural Sciences, Fuzhou, 350013, PR China
[b] School of Agriculture and Food, the University of Melbourne, Victoria, 3010, Australia
[c] Sustainable Agriculture Sciences, Rothamsted Research, Devon, EX20 2SB, UK
[d] School of Geography Sciences, Nanjing Normal University, Nanjing 210023, PR China
[e] Institute of Plant Ecology, Justus-Liebig University Giessen, Heinrich-Buff-Ring 26, 35392 Giessen, Germany
[f] School of Biology and Environmental Science and Earth Institute, University College Dublin, Belfield, Dublin, Ireland

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ABSTRACT

The effectiveness of rice-straw incorporation to alleviate environmental deterioration and increase soil fertility is widely accepted, whereas, the effect of this management on stimulating soil nitrogen (N) transformation is not fully understood. This study was conducted to investigate the effect of rice-straw incorporation on soil N transformation. An incubation experiment was conducted with rice-straw incorporated at rates of 0 (RS0), 1.67 (RS1), 3.33 (RS2) and 6.67 g kg^{-1} soil (RS3). Tracing experiments with $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ was conducted in the first (Week 1) and tenth week (Week 10) after straw incorporation, and a numerical model was used to calculate gross rates of N transformations. Incorporation of rice-straw increased gross rates of soil organic N mineralization, ammonium ($NH_4{}^+$) and nitrate ($NO_3{}^-$) immobilization and oxidized organic-N to $NO_3{}^-$, by 0.2–1.7 times, 4.6–11.6 times, 20.4–74.9 times and 6.2–20.3 times, respectively. However, the stimulation of soil N transformation via rice-straw incorporation was insignificant by week 10. Over the incubation period, the stimulation of soil inorganic N production pathways (organic N mineralization and oxidized organic-N to $NO_3{}^-$) via rice-straw incorporation was less than on consumption pathways ($NH_4{}^+$ and $NO_3{}^-$ immobilization), leading to soil inorganic N supply capacity decreasing with straw incorporation rates. Dissimilatory $NO_3{}^-$ reduction to $NH_4{}^+$ was stimulated by rice-straw incorporation, as observed in both the first and tenth week. Compared with RS0, autotrophic nitrification decreased by 14%, 25% and 46% in RS1, RS2 and RS3, respectively, but this effect disappeared by week 10. However, nitrification capacity (*NC*, the ratio of autotrophic nitrification rate to total mineralization rate) was constrained following rice-straw incorporation both in the first and tenth weeks. Decreasing autotrophic nitrification was the most important factor contributing to decreased $NO_3{}^-$ content with straw incorporation, followed by increasing $NO_3{}^-$ immobilization. The gross rate of autotrophic nitrification was negatively correlated with $NH_4{}^+$immobilization, indicating that autotrophic nitrification inhibition may be attributed to increased $NH_4{}^+$ immobilization. Therefore, based on the observations of this study, rice-straw incorporation is recommended for reducing nitrification capacity and reducing risks of N losses in subtropical acid soil.

1. Introduction

To meet increasing grain demand, chemical fertilizers are used to improve productivity in agricultural ecosystems (Wang et al., 2018). However, overuse of chemical fertilizers can lead to multiple environmental issues, such as soil acidification, low fertilizer use efficiency, and groundwater contamination (Ju et al., 2006; Wang et al., 2018). To alleviate environmental deterioration and improve soil fertility, incorporating crop straw to cultivated soils has been widely adopted in China in recent years (Gai et al., 2019). This is due to a new policy of reducing chemical fertilizers by partial replacement with organic fertilizers. Previous studies have reported the effects of straw incorporation on soil organic N mineralization (Takahashi et al., 2003; Thomsen and Sørensen, 2006), $NH_4{}^+$ immobilization (Corbeels et al., 2000; Cao

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[*] Corresponding author.
E-mail addresses: zys3505@126.com (Y. Zhang), hongding@china.com (H. Ding).

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Table 1
Soil properties before and after incubation.

	Before incubation	After incubation (Week ten)			
		RS0	RS1	RS2	RS3
pH	5.31	4.93 ± 0.05c	5.01 ± 0.10c	5.18 ± 0.17b	5.48 ± 0.11a
SOC ($g\,kg^{-1}$ soil)	13.0	13.3 ± 0.08c	13.7 ± 0.13b	14.1 ± 0.39b	14.6 ± 0.03a
TN ($g\,kg^{-1}$ soil)	1.05	1.03 ± 0.00a	1.04 ± 0.01a	1.05 ± 0.02a	1.10 ± 0.02a
NH_4^+ ($mg\,kg^{-1}$ soil)	23.8	2.60 ± 0.55a	2.91 ± 0.04a	3.55 ± 0.56a	2.24 ± 0.56a
NO_3^- ($mg\,kg^{-1}$ soil)	28.5	42.9 ± 2.72a	33.3 ± 3.18b	23.4 ± 2.36c	9.29 ± 0.56d
Olsen P ($mg\,kg^{-1}$ soil)	126	122 ± 4.33ab	129 ± 4.28a	126 ± 6.01a	112 ± 4.83b
Av. K ($mg\,kg^{-1}$ soil)	118	150 ± 9.41d	165 ± 13.9c	208 ± 6.48b	335 ± 0.12a

Where, Av. K is available potassium, SOC is soil organic C, and TN is total N. Letters indicate significant differences between plants within soils ($p < 0.05$; ANOVA with Duncan's test, *PASW Statistics 18.0*). RS0, RS1, RS2 and RS3 are rice-straw incorporated at 0.00, 1.67, 3.33 and 6.67 $g\,kg^{-1}$ soil (DWE).

et al., 2018), nitrification (Liu, 2002; Thomsen and Sørensen, 2006), dissimilatory NO_3^- reduction to NH_4^+ (Lu et al., 2015), or NO_3^- immobilization (Shindo and Nishio, 2005). However, few studies have been conducted to investigate the simultaneous N transformations which drive soil N availability following straw incorporation, which are required to understand the mechanisms behind straw incorporation effects on N availability and loss risk.

Straw incorporation may change microbial community composition and influence N transformation dynamics and N availability (Bird et al., 2003; Ryals et al., 2014). As a main pathway of NH_4^+ production, N mineralization would be improved by the incorporation of exogenous materials (Thomsen and Sørensen, 2006). Additionally, because of the positive effect of straw incorporation, the mineralization of native soil organic matter would be promoted (Muhammad et al., 2007). Soil N availability are governed by soil N mineralization and immobilization processes (Huygens et al., 2007). Generally, incorporation of straw with a large C:N ratio would improve N immobilization and cause N limitation (Bhogal et al., 1997; Beaudoin et al., 2005; Zhao et al., 2018a). However, most studies focus on the net rates of soil N transformation after straw incorporation (Khalil et al., 2005; Muhammad et al., 2007; Cao et al., 2018; Gai et al., 2019), which does not provide a good understanding about the gross rates of N transformation. The gross rates of N transformation, associated with individual processes, can elucidate the mechanisms involved in the soil N cycling (Whitehead, 2000; Müller et al., 2007).

In subtropical regions of China, which are characterized by high precipitation, NO_3^- leaching losses occur readily, while NH_4^+ is less mobile due to inhibition of ammonia volatilization caused by low soil pH (Zhang et al., 2013) and it is charged making it more susceptible to soil adsorption processes. Thus, NO_3^- is more difficult to immobilize than NH_4^+ (Nishio et al., 2001). However, crop straw incorporation can accelerate the assimilation of NO_3^- at soil water holding capacity (WHC) of 35–46% (Nishio et al., 2001). Additionally, as a main pathway of NO_3^- production in agricultural soil, autotrophic nitrification may be inhibited by straw incorporation (Zhang et al., 2012; Wang et al., 2015). Thus, adjusting the soil C:N ratio and increasing the soil microbial biomass C via straw incorporation could help reduce NO_3^- losses (Said-Pullicino et al., 2014). Others have reported that straw incorporation could reduce NO_3^- leaching losses from soil (Yang et al., 2018; Wang et al., 2019). However, it has been reported that organic matter has a positive stimulatory effect on the community and function of ammonia-oxidizers (Kalvelage et al., 2013; He et al., 2007), and that the increased dissolved organic C concentration associated with straw incorporation could increase the potential nitrification rates (Liu et al., 2016), which could improve the risk of NO_3^- loss. Therefore, to explain this phenomenon, the effects of straw incorporation on soil NO_3^- production and consumption should be considered.

We hypothesized that rice-straw incorporation influences soil inorganic N availability and its loss risk by affecting soil N transformation rates. Thus, a ^{15}N incubation experiment was conducted with rice-straw incorporation at different rates, and a ^{15}N tracing method was applied to quantify gross N transformation rates. The aims of this study were: (1) to investigate the effects of rice-straw incorporation on inorganic N production and consumption; and (2) to identify the effects of rice-straw incorporation on nitrification for risk of NO_3^- loss estimations. The results will help guide the recommendations of suitable practices for straw incorporation in subtropical acid soils.

2. Materials and methods

2.1. Soil samples

Soils were collected from a paddy site of the Fujian Academy of Agricultural Sciences, Fuzhou China (26°13′31″N, 119°04′10″E). The mean annual temperature and precipitation (thirty years average) in this region is 19.5 °C and 1350 mm. According to World Reference Base for Soil Taxonomy (Nachtergaele et al., 2000), the soil is an Anthrosol developed from granite. Topsoil (to a depth of 15 cm) was collected before fertilization in May 2018. Plant residues and roots in soil were removed after sampling. The soil was sieved to < 2 mm and air dried to 30% H_2O ($g\,g^{-1}$ dry basis). Soil samples were stored in the dark (at 4 °C) prior to use and soil properties measured before the incubation experiment began (Table 1).

2.2. ^{15}N tracing experiment

In this study, rice-straw was incorporated with soil at the following rates: 0, 1.67, 3.33 and 6.67 $g\,kg^{-1}$ dry weight equivalent soil (DWE; incorporation rates were equal to straw additions of 0, 3.76, 7.52 and 15.03 $t\,ha^{-1}$), henceforth referred to as RS0, RS1, RS2, and RS3 respectively. The C, N, phosphorus (P), and potassium (K) concentrations of the rice-straw were 232.0, 5.1, 1.6 and 20.8 $g\,kg^{-1}$, respectively. Moist soil (~30% moisture; equivalent to 30 g DWE) was weighed into 48 flasks, and rice-straw was incorporated (fully mixed) into soil after grinding and sieving. It has been reported that the effects of straw incorporation on inorganic N availability was focused in the first three months after its application (Nagarajah et al., 1989; Lou et al., 2007; Fu et al., 2012). Therefore, in this study, N transformation rates were measured in the first (24 flasks) and tenth (the other 24 flasks) week after straw incorporation.

The soil water content was adjusted to 60% of WHC and the soils were incubated in the dark at 25 °C. Samples were weighed every two days and water added as required. To allow aeration of the soil the incubation vessels were unsealed for one hour every two days. The procedure of Müller et al. (2007) was used to determine the gross rates of N transformation. ^{15}N solutions (1 ml) were added to the soil in weeks 1 and 10, this solution was accounted for in determining the soil water requirements. In brief, ammonium nitrate (NH_4NO_3) was applied to 12 flasks (comprising 30 mg NH_4^+-N kg^{-1} DWE soil and 30 mg NO_3^--N kg^{-1} DWE soil); $^{15}NH_4NO_3$ (9.75 atom% excess) was applied to 12 flasks for each treatment, and $NH_4{}^{15}NO_3$ (9.88 atom% excess) was applied to the other 12 flasks. Destructive sampling was conducted at 0.5, 48, 96, and 144 h following NH_4NO_3 application. Six flasks of each treatment (three $^{15}NH_4NO_3$ and three of $NH_4{}^{15}NO_3$) were selected randomly to measure the concentration of $NH_4{}^+$ and $NO_3{}^-$ and their ^{15}N enrichments.

2.3. Soil properties

Soil pH was determined on a soil:water mix of 1:5 (v:v) using a pH detector (DMP-2 mV, GW 1996). Soil organic C (SOC) was analyzed via wet digestion with H_2SO_4-$K_2Cr_2O_7$, total N was measured using a semi-micro Kjeldahl digestion with catalysts (Se, K_2SO_4 and $CuSO_4$), and soil available potassium (Av. K) was determined by the 1 M CH_3COONH_4 extraction method (Lu, 2000). Exchangeable $NH_4{}^+$ and $NO_3{}^-$ were extracted using 2 M KCl and shaken at 250 rpm for 60 min. After filtering, the concentration of $NH_4{}^+$ and $NO_3{}^-$ were measured using a segmented-continuous flow analyzer (Skalar, Breda, The Netherlands). The isotopic composition of $NO_3{}^-$ and $NH_4{}^+$ was determined according to the modified micro-diffusion method (Zhang et al., 2017).

2.4. Data and statistical analyses

Gross N transformation rates were calculated according to the model of Müller et al. (2007). The processes in this model are: (1) labile organic-N mineralization (M_{Nlab}); (2) recalcitrant organic-N mineralization (M_{Nrec}); (3) immobilized $NH_4{}^+$ to labile organic-N (I_{NH4_Nlab}); (4) immobilized $NH_4{}^+$ to recalcitrant organic-N (I_{NH4_Nrec}); (5) oxidized $NH_4{}^+$ to $NO_3{}^-$ (autotrophic nitrification, O_{NH4}); (6) oxidized organic-N to $NO_3{}^-$ (heterotrophic nitrification, O_{Nrec}); (7) dissimilatory $NO_3{}^-$ reduction to $NH_4{}^+$ (*DNRA*); (8) immobilized $NO_3{}^-$ to organic-N (I_{NO3}); (9) adsorption of $NH_4{}^+$ on cation exchange sites (A_{NH4}); (10) release of adsorbed $NH_4{}^+$ from cation exchange sites (R_{NH4}). The gross rates of N transformations were calculated for the first and tenth weeks and the average rates from these sampling periods were used for this study.

Curve-fitting and bivariate correlation analyses methods were used to examine the correlations between N transformation rates, soil properties, and rice-straw incorporation rates. Because of the high number of iterations in the ^{15}N tracing model, statistical tests were inappropriate for results comparison (Yoccoz, 1991). Therefore, the parameter results were analyzed via standard deviations (Müller et al., 2009). In brief, there are three cases of 95% confidence intervals to distinguish: 1) the parameters are not different when standard deviations overlap; 2) there is no overlap between standard deviations, whereas, 95% confidence intervals overlap, which indicates no significantly difference between parameters; 3) there are significant differences between parameters if 95% confidence intervals do not overlap. Duncan's test was used for multiple comparisons between different treatments (and differences noted as significant where $p < 0.05$).

The total rates of organic N mineralization (*TM*) and total immobilization of $NH_4{}^+$ (*TI*) rates were calculated as:

$$TM = M_{Nrec} + M_{Nlab} \tag{1}$$

$$TI = I_{NH4\_Nrec} + I_{NH4\_Nlab} \tag{2}$$

The nitrification capacity (*NC*, the ratio of autotrophic nitrification rate to total mineralization rate) was calculated as:

$$NC = O_{NH4} / (M_{Nrec} + M_{Nlab}) \tag{3}$$

The gross rates of inorganic N production (*NP*), consumption (*NI*) and the net rate of inorganic N supply (*NS*), were calculated as:

$$NP = M_{Nrec} + M_{Nlab} + O_{Nrec} \tag{4}$$

$$NI = I_{NH4\_Nrec} + I_{NH4\_Nlab} + I_{NO3} \tag{5}$$

$$NS = NP – NI \tag{6}$$

3. Results

3.1. Soil properties

Following incorporation of rice-straw by the tenth week, there was a positive relationship between increased pH and greater rice-straw incorporation, particularly for RS2 and RS3, with significantly greater pH than the other treatments (Table 1). Compared to RS0, SOC was increased by 3.2, 5.7 and 9.4% in RS1, RS2 and RS3, respectively. Although a general trend for increased TN concentration with greater rates of rice-straw incorporation was observed, this was not significant (Table 1). Soil $NO_3{}^-$ decreased by 22.5, 45.5 and 78.4% in RS1, RS2 and RS3 relative to RS0, respectively. After the incubation $NH_4{}^+$ concentrations were much reduced relative to the background soil concentration prior to the incubation (Table 1).

3.2. Concentrations and enrichment of $NH_4{}^+$ and $NO_3{}^-$ during incubation

During the first week after rice-straw incorporation and NH_4NO_3 addition, $NH_4{}^+$ concentrations in all treatments decreased with

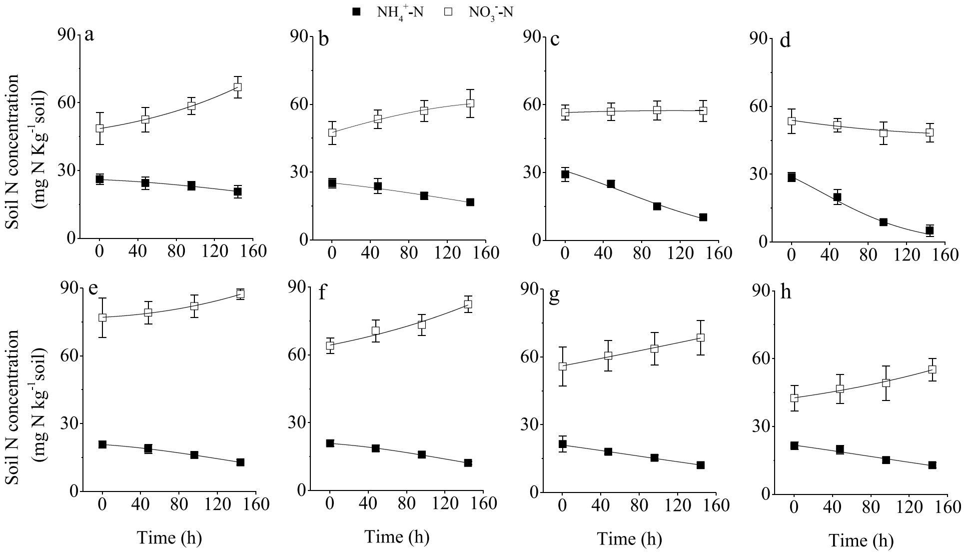

Fig. 1. Nitrogen pools in the first and tenth week following straw incorporation at different rates of straw addition, RS0 (a, e), RS1 (b, f), RS2 (c, g) and RS3 (d, h). Where, RS0, RS1, RS2 and RS3 are rice-straw incorporated at 0.00, 1.67, 3.33 and 6.67 g kg^{-1} soil (DWE). Data points present average values ($n = 6$) ± SE, under $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ treatments.

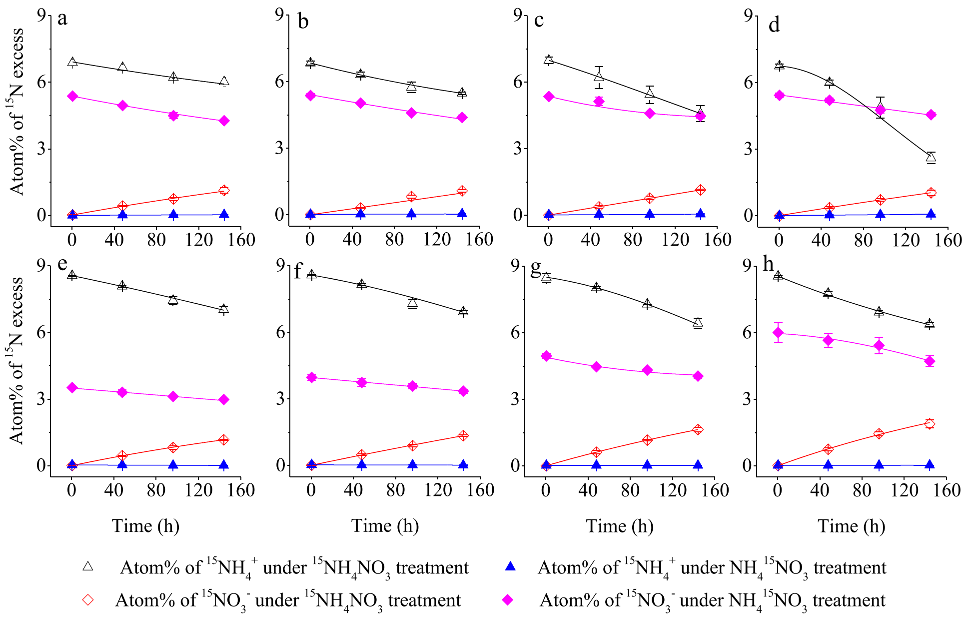

Fig. 2. Measured atom% of ^{15}N excess of NH_4^+ and NO_3^- pools of RS0, RS1, RS2 and RS3 in week 1 (a, b, c, d) and week 10 (e, f, g, h) after rice-straw incorporation. Where, RS0, RS1, RS2 and RS3 are rice-straw incorporated at 0.00, 1.67, 3.33 and 6.67 g·kg^{-1}soi.The atom% of ^{15}N excesses were the average ^{15}N enrichment of NH_4^+ or NO_3^- in soils under $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$ treatments (n = 3).

incubation time, and the slope increased with straw incorporation rates (Fig. 1a–d). Nitrate concentration increased with incubation time in RS0 and SR1, and decreased with incubation time in SR3, with no marked changes observed for RS2 (Fig. 1a–d). These results suggest that the net rates of mineralization and nitrification decreased with straw incorporation rates. The ^{15}N enrichment of NH_4^+ following addition of the $^{15}NH_4NO_3$ decreased with incubation time and the slope of $^{15}NH_4^+$ enrichment increasing with straw incorporation rates (Fig. 2a–d). This indicates that the gross rate of mineralization increased with straw incorporation rates. During the tenth week, the same trends of NH_4^+ and NO_3^- concentrations were observed in all treatments over incubation time, while the initial concentrations of NO_3^- (at 0.5 h) decreased with rice-straw incorporation rates (Fig. 1e–h). The same trends of ^{15}N enrichment of NH_4^+ and NO_3^- following addition of the $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$ were observed between different treatments (Fig. 2e–h), suggesting that gross rates of mineralization and nitrification were not markedly different in various treatments.

3.3. Gross N transformation rates

The numerical model analysis showed that, in the first week, rice-straw incorporation increased *TM*, *TI*, I_{NO3} and O_{Nrec} by 0.2–1.7 times, 4.6–11.6 times, and 20.4–74.9 times, respectively; with significantly greater transformation rates observed for RS3 than for other treatments (Fig. 3a–d). However, these differences were reduced by week 10 (Fig. 3a–d). Rice-straw incorporation stimulated DNRA as observed in both the first and tenth week, although its rate was low (Fig. 3f). There was a significant positive relationship between the rate of mineralization and NH_4^+ immobilization ($p < 0.01$, Fig. 4). Oxidation of NH_4^+ to NO_3^- was inhibited by straw incorporation in the first week, decreasing by 14, 25 and 46% in RS1, RS2 and RS3 relative to RS0, respectively (Fig. 3e), although there was not significant difference among different treatments for O_{NH4} was in week 10. However, the *NC* declined relative to RS0 in the first and tenth weeks, decreasing exponentially with increasing rates of rice-straw incorporation ($p < 0.01$, Fig. 5a). The ratio of autotrophic nitrification to NH_4^+ immobilization (*N/I*) decreased with increasing rates of rice-straw incorporation in both the first and tenth weeks ($p < 0.05$ for both, Fig. 5b). Additionally, a significant negative linear relationship between O_{NH4} and *TI* was found in this study ($p < 0.01$, Fig. 6).

To explain the variation of inorganic N concentrations during the ten weeks incubation, average gross rates of N transformations were calculated according to the rates derived in the first and tenth week. There were positive correlations between *TM* and soil pH, SOC and TN concentrations (r^2 = 0.997, 0.966 and 0.993, respectively). A significant positive linear relationship was found between average O_{NH4} and the NO_3^- concentration in soil after ten weeks incubation ($p < 0.01$, Fig. 7a). A negative linear relationship was found between average I_{NO3} and NO_3^- concentration in soil after ten weeks incubation ($p < 0.05$, Fig. 7b). The slope of average O_{NH4} relative to soil NO_3^- concentration was 60.5, which was greater than that of average I_{NO3} (24.3, Fig. 7), indicated that decreasing soil NO_3^- concentration following rice-straw incorporation was attributed firstly to O_{NH4} decreasing and subsequently I_{NO3} increasing. Additionally, both the rates of inorganic N production (*NP*) and consumption (*NI*) increased with increasing rates of rice-straw incorporation and *NS* decreased with the increasing rates of rice-straw incorporation (Fig. 8). Consequently, the concentration of inorganic N in soil decreased significantly with the *NS* ($p < 0.01$, Fig. 9).

4. Discussion

4.1. Mechanisms of soil N available affected by rice straw incorporation

The concentration of inorganic N decreased with increasing rates of rice-straw incorporation (Table 1), in accordance with previous studies of Zhao et al. (2018b), who suggested that organic material incorporation reduced net mineralization rates. The reduction of soil inorganic N concentration following rice-straw incorporation indicates that soil N supply may be reduced, which may have a negative impact on crop growth in the early stages (Chapman, 1997). However, the two main pathways of inorganic N production in soil, mineralization and oxidization of organic-N to NO_3^-, were stimulated by rice-straw incorporation in the first week (Fig. 3a, d). Whilst, the gross rates of both *NP* and *NI* increased linearly with rice-straw incorporation, during the incubation (Fig. 8). The slope of *NI* related to rice-straw incorporation was much greater than that of *NP* (Fig. 8), indicating that the stimulation of rice-straw on *NI* was greater than that on *NP*. Consequently, *NS* decreased with increasing rates of rice-straw incorporation ($p < 0.01$, Fig. 8). The concentration of inorganic N in soil increased significantly with the *NS* ($p < 0.01$, Fig. 9), leading to reductions in inorganic N availability decreasing with increasing rates of rice-straw incorporation (Table 1). These results indicated that the incorporation of rice-straw stimulated greater *NI* than *NP,* which is an important factor in the

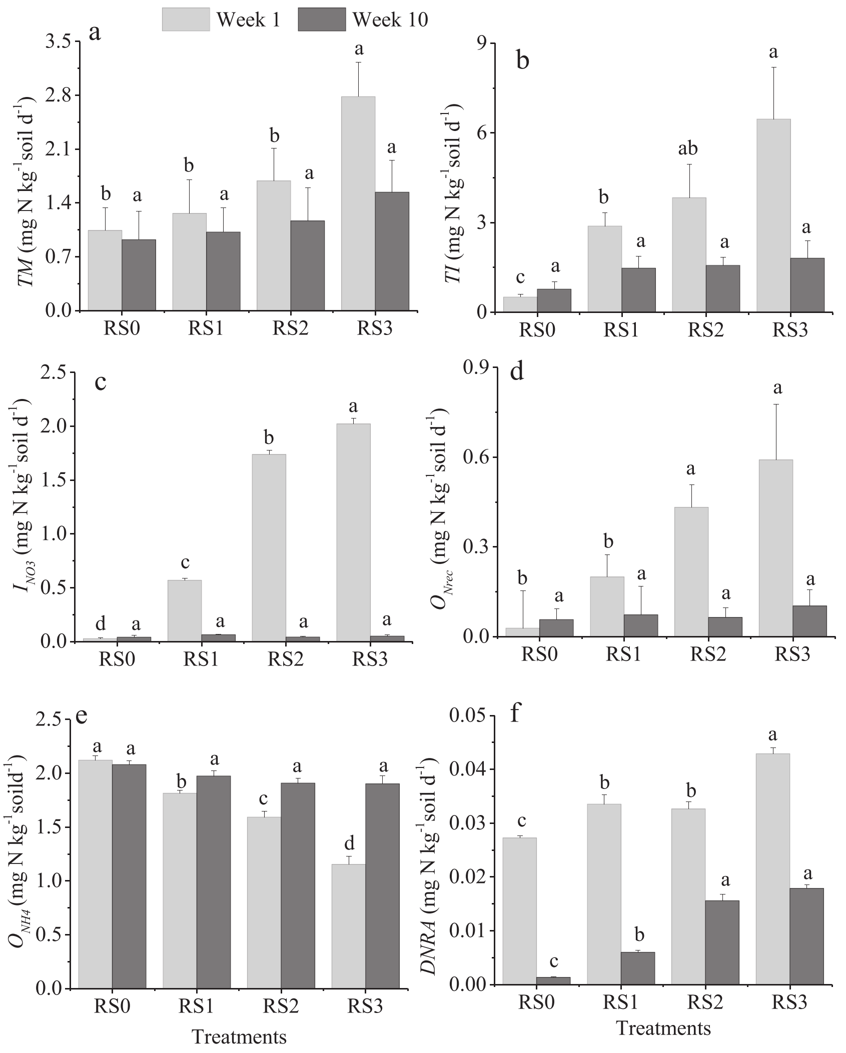

Fig. 3. Temporal variation of gross N transformation rates in soil simulated by the ^{15}N trace model. Where, RS0, RS1, RS2 and RS3 are rice-straw incorporated at 0.00, 1.67, 3.33 and 6.67 g kg^{-1} soil, Week 1 and Week 10 are first week and tenth week after rice-straw incorporation. *TM* is mineralization of organic N to NH_4^+, *TI* is immobilization of NH_4^+ to organic N, I_{NO3} is immobilization of NO_3^- to recalcitrant organic N, O_{Nrec} is oxidation of recalcitrant organic N to NO_3^-; O_{NH4} is oxidation of NH_4^+ to NO_3^-, *DNRA* is dissimilatory NO_3^- reduction to NH_4^+. Error bars show the standard error of the treatment mean ($n = 3$) and letters represent significant differences between values within time ($p < 0.05$).

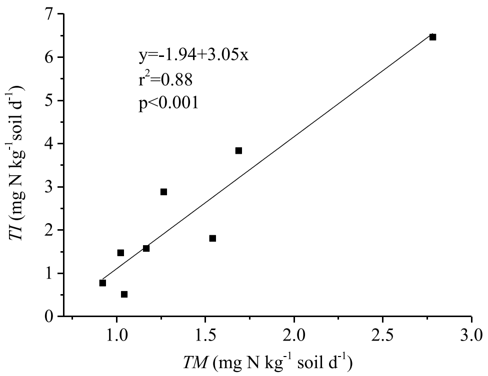

Fig. 4. The relationship between soil NH_4^+ immobilization rates (*TI*) and mineralization rates (*TM*). Data points represent mean values ($n = 3$).

dynamics of N availability. Thus, to alleviate N limitation, straw incorporation would need to be applied alongside additional N fertilizer. This finding is in agreement with Zhao et al. (2018a), who reported that rice-straw incorporation leads to N becoming a limiting factor for microbial population growth due to the increased C:N ratio caused by straw incorporation.

After incubation with rice-straw for ten weeks, average *TM* rates increased with soil pH and the concentrations of SOC and soil TN increasing. This indicates that the increases in *TM* following rice-straw incorporation could be ascribed to the associated increases in soil pH, SOC and TN concentrations (Vervaet et al., 2002; Yan et al., 2008). These results are consistent with those of Zhao et al. (2018b), who suggested that incorporation of rice-straw accelerated both *TM* and O_{Nrec} in acidic and purple soils. In our study, a significant positive relationship was found between the rate of *TM* and *TI* ($p < 0.001$, Fig. 4), indicating that the rate of mineralization is a key factor for NH_4^+ immobilization. Bengtsson et al. (2003) reported a similar phenomenon, and suggested that gross immobilization of NH_4^+ was dependent on gross mineralization.

4.2. Mechanisms of autotrophic nitrification inhibited by rice-straw incorporation

Our investigation showed that the rate of O_{NH4} was inhibited by rice-straw incorporation in the first week (Fig. 3e). These results are consistent with those of Zhao et al. (2018a), who reported that rice-straw incorporation inhibited O_{NH4} both in acidic and alkaline soils. There was a significant negative correlation between the gross rate of O_{NH4} and *TI* (Fig. 6), suggesting that competition for NH_4^+ between NH_4^+ immobilization and autotrophic nitrification processes is the key factor inhibiting O_{NH4} when rice-straw was incorporated. Deni and Penninckx (1999) suggested that this is most likely attributed to the competition between hydrocarbons in rice-straw and ammonia as substrates for ammonia monooxygenase, leading to reduced O_{NH4}. Khalil et al. (2005) suggested that this may be attributed to the slow decomposition and immobilization of N released when materials high in C are incorporated. Immobilization and nitrification are important

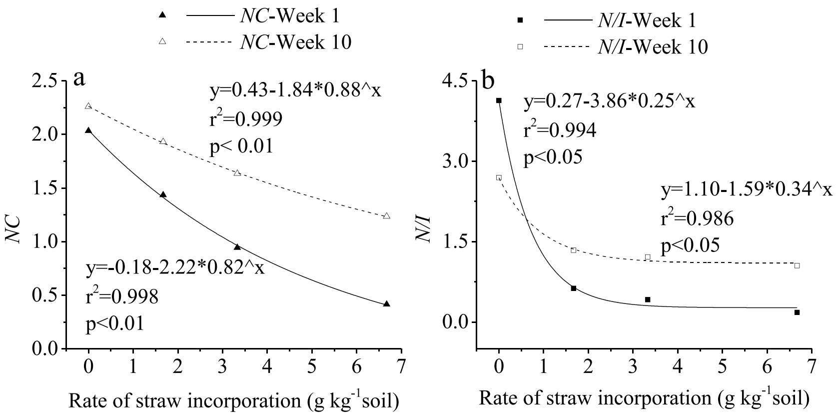

Fig. 5. The relationship between the rates of rice-straw incorporation and nitrification capacity (*NC*, a) and the ratio of autotrophic nitrification to NH_4^+ immobilization (*N/I*, b). Where, *NC* was calculated by O_{NH4}/TM, Week 1 and Week 10 are first and tenth week after rice-straw incorporation. Data points represent mean values ($n = 3$).

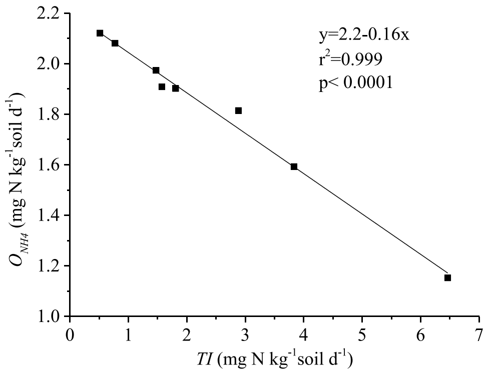

Fig. 6. The relationship between soil NH_4^+ immobilization rates (*TI*) and autotrophic nitrification rates (O_{NH4}). Data points represent mean values ($n = 3$).

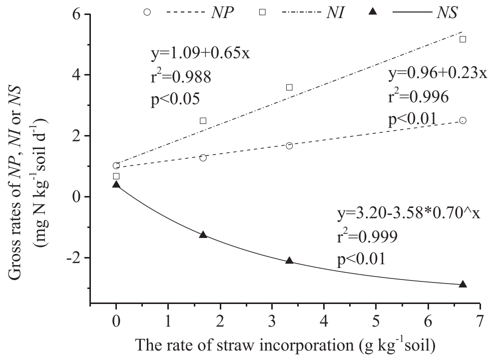

Fig. 8. The relationship between the rates of rice straw incorporation and the gross rate of *NP*, *NI* and *NS*. Where, *NP* is inorganic N production capacity, calculated by $TM + O_{Nrec}$; *NI* is inorganic N consumption capacity, calculated by $TI + I_{NO3}$; *NS* is inorganic N supply capacity, calculated by $(TM + O_{Nrec})$-$(TI + I_{NO3})$. *TM* is mineralization of organic N to NH_4^+, *TI* is immobilization of NH_4^+ to organic N, I_{NO3} is immobilization of NO_3^- to recalcitrant organic N, O_{Nrec} is oxidation of recalcitrant organic N to NO_3^-. *NP*, *NI* and *NS* were the mean rates in first week and tenth week. Data points represent mean values ($n = 6$).

pathways of NH_4^+ consumption in soil. The high C availability derived from rice-straw incorporation can support active microbial populations (Fontaine and Barot, 2005). Due to the large C:N ratio in rice-straw, microbes need to assimilate more inorganic N from the soil to meet their N demand during rice-straw decomposition (Zhao et al., 2018a). Therefore, NH_4^+ immobilization was accelerated with increasing rates of rice-straw incorporation, and nitrification is expected to be constrained due to NH_4^+ assimilation and immobilization(Chapman, 1997; Khalil et al., 2005).

Although the inhibition of O_{NH4} following rice-straw incorporation had disappeared by the tenth week, *NC* decreased with increasing rates of rice-straw incorporation both in the first and tenth week ($p < 0.01$, Fig. 5a). Additionally, the ratio of O_{NH4} to *TI* (*N/I*), which has been used as an index to investigate the likelihood for soil N losses (Stockdale et al., 2002), decreased with increasing rates of rice-straw incorporation both in the first and tenth week ($p < 0.05$, Fig. 5b). These suggest that rice-straw incorporation could reduce the risk of NO_3^- loss via leaching or runoff up until ten weeks after incorporation. In fact, our previous study found that rice-straw could reduce the rate of O_{NH4} almost until one year after incorporation, based on samples from a long-term experiment which had rice-straw incorporated into the soil every year from the previous crop at the rate of 5.2 t ha^{-1} (Zhang et al., 2015b). Similarly, Khalil et al. (2005) suggest that N losses can be reduced in acidic soils when treated with materials high in C. Therefore, incorporating straw may potentially act as a biological nitrification inhibitor and may present a promising strategy for mitigating N losses as NO_3^- (Subbarao et al., 2007; Zhao et al., 2018a).

Our observations indicated that soil NO_3^- concentration was decreased during incubation with rice-straw incorporation (Table 1). Soil NO_3^- concentration increased with the rate of O_{NH4} and decreased with increasing rate of I_{NO3} during incubation (Fig. 7), indicated that the decreasing O_{NH4} and increasing I_{NO3} were the main factors leading to

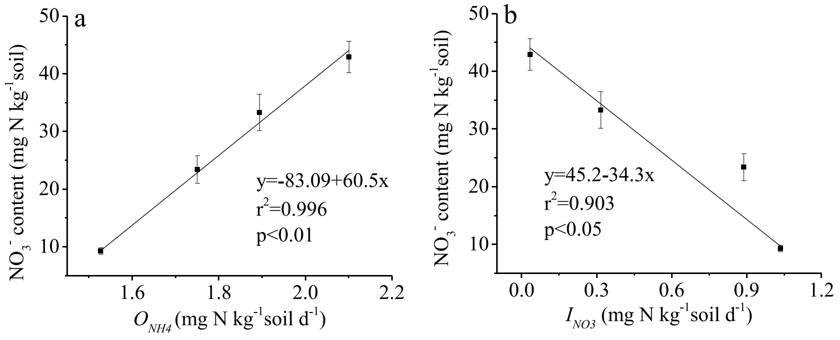

Fig. 7. The relationship between the concentration of NO_3^- in soil after rice straw incorporation ten weeks and the rates of autotrophic nitrification (O_{NH4}, a) and NO_3^- immobilization (I_{NO3},b). Where, O_{NH4} and I_{NO3} were the mean rates of autotrophic nitrification and immobilization of NO_3^- in first week and tenth week. Data points represent mean values ($n = 6$) ± SE.

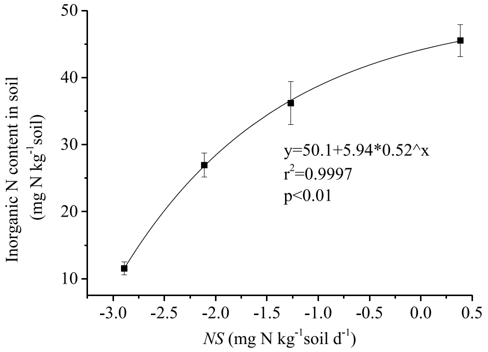

Fig. 9. The relationship between inorganic N supply capacity and inorganic N concentration in soil after incubated with rice straw incorporation ten weeks. Where, *NS* is the mean rate of soil inorganic N supply capacity calculated by the average rate of first and tenth weeks. Data points represent mean values (n = 6).

reduced NO_3^-. The slope of O_{NH4} was greater than that of I_{NO3} (Fig. 7), indicating that the O_{NH4} was more important than I_{NO3} for soil NO_3^- concentration. Additionally, the O_{Nrec} was stimulated by rice-straw incorporation in the first week (Fig. 3d), which is attributed to organic N provided by the rice-straw (Zhang et al., 2015a). While, the stimulation of O_{Nrec} following straw incorporation had disappeared in the tenth week (Fig. 3d). The relationship between rice-straw incorporation and O_{Nrec} is not fully understood. Additionally, our study was conducted under temperature and moisture (25 °C and 60% WHC) conditions optimized for microbial activity, which will differ from in-situ conditions. Soil N dynamics after rice-straw incorporation in-situ with intact soils needs further research.

5. Conclusion

Rice-straw incorporation stimulated N mineralization, NH_4^+ immobilization, oxidization of organic N to NO_3^-, NO_3^- immobilization, and dissimilatory NO_3^- reduction to NH_4^+ within the first week. Inorganic N concentration in soil was driven by soil inorganic N supply capacity, which decreased with increasing rates of rice-straw incorporation, because inorganic N consumption was greater than production. Autotrophic nitrification was inhibited by rice-straw incorporation in the first week, with no effect observed in the tenth week. However, nitrification capacity was constrained following rice-straw incorporation both in the first and the tenth week. Decreasing rates of NH_4^+ oxidation to NO_3^- was the most important factor for reduced NO_3^- concentrations, which declined with increasing rates of rice-straw incorporation, followed by immobilization of NO_3^- to organic N. The gross rate of NH_4^+ oxidation to NO_3^- was negatively correlated with immobilization of NH_4^+ to organic N, indicating that inhibition of autotrophic nitrification following rice-straw incorporation may be attributed to NH_4^+ immobilization, stimulated by rice-straw incorporation. However, further studies are needed to quantify the contribution of all factors on autotrophic nitrification inhibition.

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